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# The Impact of Astaxanthin Supplementation on the Production Performance of Laying Hens Exposed to Heat Stress

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This study was carried out in The Department of Animal Production, College of Agriculture, University of Basrah, has a poultry hall. The research spanned from November 22, 2022, to January 21, 2023, having the main goal of figuring out how laying hens' ability to produce eggs is affected by the amount of astaxanthin they consume in their diet. At the age of 60 weeks, 60 ISA Brawn laying hens were divided into five treatment groups at random, with 12 laying hens per treatment, and the birds of each treatment were divided into four replicates (3) chickens per (repeater). The birds were fed a productive diet containing 17.5% crude protein, and a representative energy of 2735.20 kcal/kg feed, for the T1, T2, T3, T4, and T5 parameters, astaxanthin was administered at amounts of 0, 3, 6, and 12 mg/kg feed, respectively. The results showed that the addition treatments T2, T3, T4, T5 to a significant superiority (P<0.05) in each of the number of eggs produced, egg production ratio, egg mass, the quantity of food consumed and improved nutritional conversion efficiency for all addition parameters compared to T1 treatment. This study discovered that feeding astaxanthin to laying hens improved their ability to produce.
**Keywords**: Astaxanthin, Production performance, Laying hens, Heat stress, Egg production.

## INTRODUCTION

The poultry sector plays a vital role in meeting human needs for animal protein, with egg production being a key product. Heat stress poses a significant challenge to laying hens, negatively impacting their health and performance. This study explores the potential of astaxanthin, a natural antioxidant, to mitigate the adverse effects of heat stress on laying hens' productivity. Astaxanthin's ability to neutralize free radicals and enhance immune performance makes it a promising alternative to industrial antioxidants. Increased temperatures may lead to a decline in production, a high rate of mortality, and a deterioration in egg quality, causing significant economic losses to farmers and industry in general (Mehaisen, *et al.*, 2019). This leads to a negative impact on the functions and physiological functioning of their vital organs as well as stimulating oxidative stress and oxidative damage through the production of free radicals (*Cornescu, et al.*, 2023). Due to this, recent investigations and studies have been inclined towards discovering alternatives and remedies to mitigate these issues. A promising avenue involves incorporating natural antioxidants into poultry diets, thereby replacing antibiotics and bolstering the impact of endogenous antioxidant enzymes in poultry (Yu, *et al.*, 2019). The ability to improve both productive and immune performance while reducing the activity and effects of free radicals is considered a positive attribute (Sandmann, 2019), These natural alternatives are considered safer than the industrial options used in poultry feed, which can cause worry among consumers of these products (Pashtetsky, *et al.*,2019). Astaxanthin, a natural carotenoid and antioxidant, has been confirmed as safe by the European Food and Safety Agency (EFSA). Its significance lies in its ability to effectively neutralize numerous free radicals generated due to oxidative stress. It inhibits their activity and protects proteins, lipids, and cell membranes from oxidation by penetrating the double-layer of the cell membrane, in contrast to other antioxidants that target specific locations either outside or inside the cell membrane (Ambati, *et al.*, 2014). To achieve these benefits and effects, the addition of astaxanthin to heat-stressed laying hens' diets represents a promising potential for improving the productive and qualitative qualities of eggs, as well as adding astaxanthin to chicken diets may be an effective strategy to improve tolerance to extreme thermal conditions and enhance the chickens' ability to adapt to environmental challenges (Magnuson, *et al.*, 2018).

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Construction In keeping with the move towards using natural antioxidants, this study sought to determine the ideal level of astaxanthin to include in the diets of laying hens by investigating the effects of varied astaxanthin intake on their productive performance.

## MATERIALS AND METHODS

The Department of Animal Production of the University of Basrah's Faculty of Agriculture conducted this study, from November 22, 2022, to January 21, 2022. It aimed to investigate the impact of adding astaxanthin in varying amounts (0, 3, 6, 9, and 12 mg per kg of feed) to the food on how productively laying chickens' function. Sixty ISA Brawn laying hens, aged 60 weeks and with an initial weight, were employed in the experiment, each weighing 178065 g, and were allocated at random over five transactions, with 12 laying hens assigned to one of the treatments. Each treatment's birds were then separated into four (3) chickens per (repeater). These were the transactions: The initial action: The control group for the first treatment (T1) lacked any astaxanthin addition. In the second treatment (T2), 3 mg/kg of feed was added. In the third treatment (T3), 6 mg/kg of feed was added. In the fourth treatment (T4), 9 mg/kg of feed was added. In the fifth treatment (T5), 12 mg/kg of feed was added. The algae Haematococcus pluvialis is the natural source of the astaxanthin used in this study, which was obtained from GO-HEALTHY. The chickens were housed in typical cages that were each 608 cm2 in size and measured 48 cm broad, 38 cm deep, and 38 cm tall. A 16-hour light, 8-hour dark cycle was used during the trial. The temperature was controlled using electric heaters and air pullers and set to 32°C to induce heat stress in the birds. Water was provided freely (Ad libitum), and crushed feed was provided daily 112 g / bird during the trial period and according to Table 1.

**Studied traits**

**Egg Production Percent:** At 2 PM every day during the trial, eggs were collected. The Hen Day Production formula (North,1984) was used to determine the egg production ratio for each hen, based on the number of chickens remaining in each group at the end of each period.

$$\text{Egg production percent} = \frac{\text{Egg production}}{\text{hen number}} \times 100$$

**Egg Weight:** The total weight of the eggs was measured weekly for each treatment group using a Mttuler 2000 balance. During each trial period, the weight of the eggs was measured to the nearest (g) and the average egg weight was computed.

**Feed intake:** Feed intake was recorded daily and calculated according to Al-Ashoor and Al-Salhie (2020).

**Feed Conversion Coefficient:** The feed conversion coefficient was determined using a specific formula (North, 1984):

$$\text{FeedConversion Coefficient} = \frac{\text{Feed intake}}{\text{Eggmass}}$$

**Egg mass:** was calculated using a specific formula (North, 1984):

$$\text{Egg mass} = \frac{\text{Egg production percent}}{100} \times \text{Eggweight mean}$$

**Table 1. The experiment's composition included the proportions of feed materials expressed as percentages.**

| Feed material | % |
|---|---|
| Yellow corn | 36.00 |
| Wheatgrass | 28.50 |
| Soybean meal (4-4% protein) | 16.00 |
| Protein | 10.00 |
| Vegetable oil | 1.50 |
| Limestone | 7.70 |
| Table salt | 0.30 |
| Total | 100.00 |
| **Chemical Analysis1** | |
| Energy represented (kcal/kg feed) | 2759.00 |
| Crude protein | 17.75 |
| Crude fiber | 3.03 |
| Lysine | 60.8 |
| Methionine + Cysteine | 0.68 |
| Calcium | 3.60 |
| Available phosphorus | 0.44 |

Estimated chemical composition using NRC (1994)

**Statistical analysis:** The effects of various treatments on the attributes under investigation were examined using a completely randomized design (CRD), and the mean differences between treatments were compared using Duncan's (1955) multiple range test with a significance level of (0.05). SPSS, (2019) was used for statistical analysis.

## RESULTS AND DISCUSSION

Table 2 findings demonstrate that there are significant differences ($P<0.01$) between the treatments in the characteristic of the number of eggs produced during the first productive period (60–63 weeks). All of the astaxanthin addition treatments—T2, T3, T4, and T5—showed a significant increase (($P<0.01$)) in comparison to treatment T1. Table 2 shows significant differences (($P<0.01$)), with treatment T5 having the greatest rates between the experimental treatments in the percentage of egg production (HD%) and the addition treatments significantly affected it. outperformed the T1 treatment and the best rate of egg production rate was recorded in the addition treatment T5. The results of Table 2 show that the addition treatments are significantly superior ($P<0.01$) On the control treatment by egg weight except for the control treatment except for the T2 treatment with which it did not differ significantly, and the best egg weight rate recorded in the T5 treatment. As for the

**Table 2. Effect of Astaxanthin Use on Production Performance of Laying hens Subjected to Heat Stress during Production Period (60-63) (Mean2± SE).**

| [1]Treatments | Control T1 | T2 | T3 | T4 | T5 | SEM | P value |
|---|---|---|---|---|---|---|---|
| Number of Eggs | 19.58$^{d}$ | 20.67$^{c}$ | 21.17$^{c}$ | 21.92$^{b}$ | 22.58$^{a}$ | 0.29 | 0.000 |
| Egg production (%) | 69.94$^{d}$ | 73.81$^{c}$ | 75.60$^{c}$ | 78.27$^{b}$ | 80.66$^{a}$ | 1.02 | 0.000 |
| Egg Weight (g) | 62.90$^{d}$ | 63.01$^{cd}$ | 63.08$^{bc}$ | 63.18$^{ab}$ | 63.26$^{a}$ | 0.04 | 0.001 |
| Egg mass (g) | 1231.86$^{d}$ | 1302.22$^{c}$ | 1335.27$^{c}$ | 1384.82$^{b}$ | 1428.92$^{a}$ | 18.75 | 0.000 |
| Feed intake (g) | 3079.33$^{d}$ | 3087.33$^{cd}$ | 3092.67$^{c}$ | 3117.33$^{b}$ | 3130.00$^{a}$ | 5.26 | 0.000 |
| Feed conversion efficiency (g/g) | 2.50$^{a}$ | 2.38$^{b}$ | 2.33$^{bc}$ | 2.26$^{cd}$ | 2.20$^{d}$ | 0.03 | 0.000 |

[1]T control diet (no addition) and 2T, T3, T4, T5: astaxanthin use treatments 3, 6, 9, 12 g/kg feed respectively. [2]* a, b: At a significance level of P0.05, the means in the same row with different letters indicate that there is a significant difference between the groups.

**Table 3. Effect of Astaxanthin Use on Production Performance of Egg Chickens Exposed to Heat Stress during Production Period (64-67) (Mean2± SE).**

| [1]Treatments | Control T1 | T2 | T3 | T4 | T5 | SEM | P value |
|---|---|---|---|---|---|---|---|
| Number of Eggs | 20.33$^{d}$ | 21.67$^{c}$ | 22.75$^{b}$ | 23.08$^{b}$ | 24.00$^{a}$ | 0.35 | 0.000 |
| Egg production (%) | 77.38$^{d}$ | 73.81$^{c}$ | 81.25$^{c}$ | 82.44$^{b}$ | 85.71$^{a}$ | 1.24 | 0.000 |
| Egg Weight (g) | 63.05$^{e}$ | 63.17$^{d}$ | 63.24$^{c}$ | 63.35$^{b}$ | 63.44$^{a}$ | 0.04 | 0.000 |
| Egg mass (g) | 1231.86$^{d}$ | 1302.22$^{c}$ | 1335.27$^{b}$ | 1384.82$^{b}$ | 1428.92$^{a}$ | 18.75 | 0.000 |
| Feed intake (g) | 3077.00$^{c}$ | 3083.00$^{c}$ | 3104.67$^{b}$ | 3115.33$^{ab}$ | 3131.00$^{a}$ | 5.79 | 0.000 |
| Feed conversion efficiency (g/g) | 2.40$^{a}$ | 2.25$^{b}$ | 2.16$^{c}$ | 2.13$^{c}$ | 2.06$^{d}$ | 0.03 | 0.000 |

Control diet (no addition) and 2T, T3, T4, T5: astaxanthin use treatments 3, 6, 9, 12 g/kg feed respectively. [2]* a, b: At a significance level of P0.05, the means in the same row with different letters indicate that there is a significant difference between the groups.

egg mass, it is noted from the results of Table 2 that there is a significant increase (P<0.01) in the egg mass for all addition treatments compared to T1 treatment. The increase was gradual with increasing astaxanthin concentration. The results show a significant increase in the feed intake for the addition treatments T5, T4 and T3 over the treatments T1 and T2 and the highest rate recorded for the addition treatment T5. The food conversion coefficient in addition treatments T5, T4, T3, and T2 improved significantly (P<0.01) as compared to the T1 treatment, and the addition treatment T5 improved significantly (P<0.01) more than the other addition treatments.

The effect of astaxanthin supplementation on the productive performance of laying hens throughout the heat stress phase (64–67 weeks) is depicted in Table 3 (see below). The findings show that there are significant differences (P<0.01) in the average number of eggs produced between the experimental groups. In comparison to the control treatment, the addition of astaxanthin led to a notable rise. T5 demonstrated the greatest improvement among the addition therapies, followed by T4 and T3, the other two therapies. When compared to the other experiment treatments, the T5 addiction treatment significantly increased (P the percentage of eggs produced, followed by the T4 treatment, which did not significantly differ from the T3 treatment, the T2 treatment, and the control treatment, which recorded the lowest rates.

The egg weight for astaxanthin addition treatments increased gradually with increasing the level of addition, as shown in Table 3, and this increase was statistically significant (P<0.01) when compared to the control. According to the findings, the addition treatments significantly increased (P<0.01) the egg mass rate, with the T5 treatment recording the greatest rates. From Table 3's findings, it can be seen that, except for T2 therapy, there is a considerable rise in feed intake in favor of more treatments. Which observed a substantial increase (P<0.01) in feed intake in Favor of the T5 treatment compared to other addition treatments but did not significantly differ from the control treatment. A considerable improvement is shown in Table 3 (P<0.01) in the nutritional conversion efficiency of all addition parameters compared to control and this improvement was gradual with an increase in the level of astaxanthin in the feed. The findings from tables 2 and 3 show that adding dietary supplements to the diets of heat-stressed laying hens astaxanthin resulted in enhanced egg production in terms of both quantity and percentage. This improvement is attributed to the role of astaxanthin in regulating the secretion of pituitary hormones, particularly FSH, which is essential for activating the growth of ovarian alveoli, and LH hormone, which facilitates the ovulation process and increases the rate of ovulation. Additionally, astaxanthin plays a vital role in synthesizing steroid hormones, namely progesterone and estrogen, by activating enzymes that aid in the synthesis of sex steroids within the ovaries (He, *et al.*,2023). Lin, *et al.*, (2021) mentioned the relationship of astaxanthin in activating the biosynthesis of steroid hormones in the ovaries which are essential for the development of ovarian alveoli. Astaxanthin's ability to improve feed digesting intake by promoting the proliferation and reproduction of helpful bacteria already present in the

stomach may account for the improvement in egg weight in favour of astaxanthin addition treatments compared to T1 control treatments microbiota and thus increasing their spread over the mucous layer that covers the intestinal cells and secretes amino acids, which form the proteins necessary for egg formation (Haasbroek, *et al.*, 2019).

The role of astaxanthin in increasing the concentration of oestrogen in the serum of the white jag may be attributed to its role in improving the number of eggs, egg production ratio, and egg weight in favour of astaxanthin addition treatments, as it was observed that there is a positive correlation between the level of oestrogen in blood serum, the egg weight, and the number of eggs lipoproteins, neutral fats, fatty acids, total protein, egg weight and percentage of its production (Novero, *et al.*, 1991).

As well as the role of astaxanthin stimulating the growth and reproduction of beneficial microorganisms that contribute to fermentation activity, this ups the output of the short-chain fatty acids that follow in a decrease in intestinal pH and thus benefit from the solubility and absorption of mineral elements such as zinc, sodium, and chloride (Vieco- Saiz, *et al.*, 2019). The increase in beneficial microbiology in the intestine on the surface of the lining mucous material has a role in reducing the speed of passage of nutrients resulting in giving a greater opportunity to digest and absorb nutrients and their exposure to proteolytic enzymes, carbohydrates, fats, and then increasing the level of utilization of them, which reflects positively on the productive performance of birds (Azad, *et al.*, 2018). Yadav and Jha (2019) pointed out that promoting beneficial revival in the intestinal flora has a role in inhibiting and excluding harmful bacteria and maintaining microbial balance in favor of beneficial bacteria that contribute fatty acid synthesis and the manufacture of antibiotics and thus stimulate immunity in birds and improve their health status and thus achieve the greatest benefit from feed.

The enhancement of beneficial bacteria in the intestinal flora provides the appropriate environment for the activity of digestive enzymes such as pepsin, which digest proteins and increase their absorption in the intestine and utilize them (Theobald, 2015).

the substantial increase in feed consumption due to additional treatments (Tables 2,3) may be because birds prefer to eat reddish-colored feed resulting from mixing with astaxanthin and lead to higher intake of feed despite heat stress (AL-Sharifi, *et al.*, 2011). A negative association between temperature rise and the rate of feed intake may be the cause of the impact of heat stress on the amount of feed consumed and for the production periods under study (Xing, *et al.*, 2019; Srankova, *et al.*, 2019). Research findings indicate that a temperature increase beyond the range of 29-35°C adversely affects feed consumption and egg production in laying hens. Moreover, the high temperature leads to a reduction in egg weight even when the amount of feed consumed is the same as chickens not exposed to heat stress. These observations strongly suggest that high temperatures directly impact the performance of laying hens. (Sahin *et al.*, 2018; Ibtisham *et al.*, 2019). The reduction in productivity is not primarily due to the impact of heat stress on feed intake however, the high temperature affects the increase in the concentration and secretion of the hormone correct none in the blood, which leads to an increase in protein catabolism, which reduces production performance as protein is the main element in egg production, and therefore can directly affect the performance of laying hens' production (Sahin *et al.*, 2018), and the decrease in the productive performance of birds in different experimental treatments in general and control treatment in particular to below the standard rates of this strain may be due to the effect of heat stress lowering thyroid hormone Triiodothyronine T3 (Ximenes, *et al.*, 2018). The high temperature leads to an increase in the appetite of birds to eat water, which reflects negatively on the reduction of the activity of digestive enzymes, which reduces the utilization of feed (Li, *et al.*, 2015).

The significant improvement in the efficiency of food conversion (Table 2,3) may be due to the benefit of birds with astaxanthin addition treatments The large rise in egg weight and egg production ratio was the cause of this improvement. Studies on laying hens revealed that astaxanthin increases the activity of advantageous microscopic cells in their digestive tract, which secretes several digestive enzymes and favorably influences food digestion and absorption, which is reflected in the improvement of the efficiency of food conversion, which leads to improved performance bird productivity (Haasbroek, *et al.*, 2019; Danson, *et al.*, 2021).

**Conclusion**: In conclusion, astaxanthin supplementation at doses of 3, 6, 9, and 12 mg/kg feed positively affects the production performance of laying hens exposed to heat stress. This natural antioxidant shows promise as an alternative to industrial options, providing a safer and effective strategy to enhance egg production and overall poultry health**.**

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